**Advances and surprises in a decade of oocyte meiosis research**

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**Abstract**

Eggs are produced from progenitor oocytes through meiotic cell division. Fidelity of meiosis is critical for healthy embryogenesis – fertilisation of aneuploid eggs that contain the wrong number of chromosomes is a leading cause of genetic disorders including Down's syndrome, human embryo deaths and infertility. Incidence of meiosis-related oocyte and egg aneuploidies increases dramatically with advancing maternal age, which further complicates the 'meiosis problem'. We have just emerged from a decade of meiosis research that was packed with exciting and transformative research. This minireview will focus primarily on studies of mechanisms that directly influence chromosome segregation.

**Introduction**

Chromosome segregation is conserved throughout eukaryotic cell division – both meiotic (gametes) and mitotic (somatic) cells divide by separating their chromosomes equally into two new cells. Several features are shared between these two modes of cell division. For instance, both programmes initiate with disassembly of the nucleus that contains the genome, a step that liberates the chromosomes into the cytoplasm. This is soon followed by assembly of a bipolar spindle machinery from microtubules that collects the newly freed chromosomes and arranges them at its centre in metaphase (Figure 1) (1-4). When all chromosomes' kinetochores are attached to a stable population of microtubule bundles (k-fibres), silencing of the spindle assembly checkpoint signals the cell's readiness to separate the chromosomes during anaphase (Figure 2A) (5-7).

In females, meiotic chromosome segregation produces fertilisable eggs from progenitor oocytes. Unlike somatic cells, the nuclei of immature oocytes contain recombined homologous chromosomes that are held together by cohesive forces, applied through the Cohesin complex (Figure 2A) (8). Each homologue is made up of sister chromatids whose centromeres are also held together tightly. Sequential

removal of cohesion proteins selectively from the chromosome arms (in anaphase I) (Figure 2B) and from the centromeres (in anaphase II) (Figure 2C) allows separation of homologous chromosomes in meiosis I and of sister chromatids in meiosis II (Figure 2). Unlike mitosis, the second meiotic division occurs without an intervening step of DNA replication, thus giving meiosis one of its identifying features, reductional division.

Errors during this beautifully complex form of cell division however result in aneuploidy, a cellular state of containing too many or too few chromosomes, and its incorrect execution in oocytes and eggs underlies pregnancy failure, infertility and trisomy births (8-10). Chromosome segregation errors in female meiosis occur more frequently than in mitotic cells and indeed in meiotic sperm cells (11-14). Furthermore, the incidence of oocyte and egg aneuploidy increases dramatically with advancing maternal age (the 'maternal age effect') (8-10).

The last decade has seen immense efforts from laboratories worldwide to decipher the reasons behind error-prone chromosome segregation in oocytes. These have brought forth stunning advances in our understanding of the inner workings of female meiosis using models ranging from yeast, starfish to human oocytes. This mini-review will highlight, in no particular order of importance or preference, some of the key studies (with focus on mammalian systems) that either answered long-standing questions in the field or made unexpected discoveries, only to remind us that female meiosis is even more special than we had ever thought.

**1. Cohesion loss emerges as a maternal age risk factor**

It would seem that age-dependent deterioration of the forces that bind chromosomes together is the most obvious oocyte aneuploidy risk factor. However, it remained unclear whether chromosome cohesion weakens with advancing maternal age in mammalian oocytes. The 2010's set off with the discovery that it does. Two independent studies reported this phenomenon through comprehensive examination of chromosomes in oocytes obtained from young and old mice (15, 16). Unlike in young oocytes, sister centromeres in oocytes from older animals were found to be farther apart, which indicated weakened cohesion. This was confirmed by presence of reduced levels of Rec8, a meiosis-specific component of the Cohesin complex, on chromosome arms and centromeres of old oocytes. Age-dependent depletion of

cohesion was also shown to disrupt the bivalent structure of chromosomes and lead to premature separation of sister chromatids, which together can explain how most aneuploidies arise in old eggs. This finding was a major contribution to the field and laid the foundation for several other excellent studies of the causes of maternal age-related oocyte aneuploidy.

**2. Mechanical fusion of sister kinetochores underpins their co-orientation**

Meiosis II division is often likened to mitosis – they both serve to separate sister chromatids. Indeed, while embryo aneuploidies can be traced back to errors in either round of meiotic division, meiosis I is more unique – homologues are separated while replicated sisters are kept together. This generally works because sister kinetochores co-orient in meiosis I (they face the same spindle pole). Such co-orientation, and thus co-migration at anaphase, was proposed to require fusion between sister chromatids' kinetochores. Direct evidence for this came from perhaps the most mechanistic study of meiosis throughout the decade (17). In this study, functional meiotic kinetochores that are able to processively track depolymerising microtubule ends were purified from yeast cells arrested in metaphase of meiosis I. *In vitro* force measurements revealed that meiotic sister kinetochores form several piconewtons stronger attachments to microtubules than meiosis II or mitotic non-sister kinetochores, which suggested they are fused. Purified sister kinetochores also contain more microtubule binding elements, which explains their increased binding capacity and further supports the fusion hypothesis. Finally, a series of genetic experiments combined with *in vitro* assays showed that the Monopolin complex mechanically crosslinks sister kinetochores – its disruption lowers microtubule attachment strengths of meiosis I sister kinetochores to significantly weaker and mitosis-like levels, which ultimately prevents their co-orientation. Interestingly, although Monopolin itself is not conserved in mammals, the fusion of sister kinetochores is conserved throughout various species (18-21). Importantly, we now know sister kinetochore fusion could be a maternal age-dependent aneuploidy risk factor in human oocytes (highlighted next). This study also demonstrated strong need in the oocyte meiosis field for mechanistic *in vitro* reconstitution assays that have profoundly accelerated discoveries in the mitosis field.

### 3. Split kinetochores confuse the meiotic spindle in aged human oocytes

Technological advances in the last decade, high-resolution microscopy in particular, have enabled visualisation of meiotic chromosome segregation mechanisms at unprecedented details. Cell biologists in the field have also benefited immensely from scientific policies that are permissive to research using human oocytes as models of female meiosis. As a result, we have learned a great deal about increased susceptibility of human oocytes to cohesion weakening from studies that provided new insights into the genesis of human aneuploidy. Two of these studies extensively examined the configurations of human oocyte chromosomes, spacing between their kinetochores and their k-fibre attachment modes in fertility treatment patients of various age groups (22, 23). Unexpectedly, sister kinetochores in meiosis I were found to split apart with advancing maternal age (Figure 3A). On some sisters, kinetochores are so far apart that they function as separate microtubule-binding entities that associate with distinct k-fibres (Figure 3A). This is further complicated by the unique geometry of human oocyte chromosomes – the near square shape of some bivalents means that inter- and intra-kinetochore distances can sometimes be so similar that the meiotic spindle is unable to distinguish sisters from non-sister kinetochores (22). This strikingly can lead to sister kinetochore biorientation in meiosis I that, in theory, invariably causes separation of sister chromatids in anaphase I (Figure 3B). These meiotic phenotypes could be exaggerated in human oocytes that are deemed unsuitable for IVF treatment. Notwithstanding this, these findings have arguably demonstrated the most direct implication of failing to tightly hold sister kinetochores together in meiosis I (17).

### 4. Beyond cohesion: defective microtubules build poor spindles in old oocytes

By the second half of the decade, emergence of chromosomal cohesion as a major factor in oocyte aneuploidy had mobilised many in the field to this area of meiosis research. One study, however, broke trend and explored aneuploidy risk factors in aged oocytes that were, strictly speaking, unrelated to chromosomes. This led to the discovery that oocyte ageing is accompanied by defects in microtubule dynamics that induce multipolar spindle intermediates and increase the likelihood of incorrect chromosome-microtubule attachments (24). Importantly, defective spindle dynamics are independent of weakened chromosomal cohesion – false spindles automatically assemble in young oocytes in the absence of a nucleus but generally fail to form in old

oocytes that do not contain a nucleus. Ultimately, that it is not all about cohesion was nicely demonstrated by nuclear transfer experiments where old oocyte nuclei and their contents were placed inside the cytoplasm of young oocytes and vice versa. After nuclear envelope disassembly, young oocytes with normal microtubule dynamics assemble bipolar spindles that collect and align old oocyte chromosomes. However, defective microtubules in old oocytes transiently build multipolar spindles that promote erroneous kinetochore-microtubule attachments and eventually fail to align young chromosomes, which are presumably still held together tightly. This exemplary study coupled classic micromanipulation techniques with quantitative live oocyte imaging to provide new and plausible explanation for genesis of chromosomal non-disjunction in aged oocytes. It also underscored the importance of carefully executed disruptive studies in moving the oocyte meiosis field forward.

**5. Actin replaces microtubules for long-range transport in oocytes**

Given the distinct organisation of cytoplasmic actin filaments in oocytes, it is perhaps unsurprising that the actin cytoskeleton performs new functions in these large cells. However, when it was discovered in 2011 that long-range vesicle transport – a classically microtubule-dependent process – is exclusively driven by actin filaments in oocytes (25), molecular details of a previous discovery where actin substitutes for microtubules in meiotic spindle positioning were only starting to surface (26-28). In oocytes, vesicles were declared no longer passive cargoes of motors – they actively participate in laying down their own transport tracks by recruiting the actin nucleators Formin-2, Spire-1 and Spire-2 (25). Vesicle-derived actin filaments were shown to be interconnected all the way to the plasma membrane and, motorised by Myosin-Vb, vesicles travelled along them to reach the cell surface. In this intricate web of actin, vesicles move randomly as individuals but head for the cell surface as a collective. It was later shown that vesicles not only assemble the actin network but also dynamize it for effective transport of the meiotic spindle to the cell surface (29). The path to this discovery was filled with transformative high-resolution live imaging technologies, which have advanced even further since. The work itself has inspired fans of live cell imaging and proven mammalian oocytes as one of the best models to study cytoskeletal organisation.

## 6. Ready or not: Chromosomal actin patches block untimely microtubule access

The oocyte size problem in mice is aggravated in starfish oocytes, but these giants have evolved to use actin as a coping mechanism. In the decade that preceded, they were found to assemble a fishnet of actin filaments to collect chromosomes scattered throughout the nucleus (30), which itself is nearly as large as a mouse oocyte. At a time when the significance of cytoskeletal crosstalk is reaching new heights in cell biology (31), how actin-driven chromosomal collection is coordinated with kinetochore-microtubule capture in these large cells remained a mystery. As it turns out, a classic mechanism that resembles chromatin-dependent microtubule nucleation is the key. After nuclear envelope breakdown, the Arp2/3 complex nucleates actin around the chromosomes in a Ran-GTP-dependent manner (32). These actin patches buy oocytes enough time to collect all scattered chromosomes by preventing kinetochore-microtubule attachments in the 5 minutes after nuclear envelope breakdown. When actin patches are depolymerised, microtubules begin to capture nearby chromosomes immediately after nuclear envelope breakdown. Interestingly, the oocyte actin network is permissive to movement of prematurely captured chromosomes – but these are pulled and squeezed through it and eventually lead to its local collapse. Because contraction of this network normally transports chromosomes to the assembling microtubule spindle (30), its local collapse can undermine collection of distally positioned chromosomes. Finally, kinetochore capture is highly coordinated in starfish oocytes – once all chromosomes are transported, their actin patches are disassembled to allow synchronous kinetochore-microtubule attachments. Functional cooperation between globally and locally nucleated actin filaments was beautifully demonstrated in this study (32), which has brought the role of actin even closer to the ultimate subjects of meiosis, the chromosomes. Along with other seminal works of the decade, it has placed the actin cytoskeleton at the centre of mechanisms that prevent oocyte aneuploidy.

## 7. Spindle actin prevents oocyte aneuploidy

The 70's and 80's must have been an exciting time for those campaigning for actin's function in spindles – several reports of spindle actin presence fuelled the idea that it might participate or even drive chromosomal separation. However, many of these were pronounced sample preparation artefacts and it was assumed that spindles in animal cells are generally devoid of actin. This notion was emphatically resurrected in

the 2000's with evidence coming from models such as flies, mice, frogs and yeast (33). Phalloidin labelling in fixed non-manipulated mouse oocytes unequivocally showed prominent actin filaments in animal spindles (27). We now know that actin filaments progressively incorporate into newly built meiotic spindles and are present in oocytes of several mammalian species including humans (34). Genetic and pharmacological loss-of-function assays conclusively showed that spindle actin is critical to avoid oocyte and egg aneuploidy. Oocyte spindles that do not contain actin fail to efficiently align and equally segregate chromosomes. Acute actin removal from normally built spindles causes some properly aligned chromosomes to pop out of alignment and travel to the spindle pole where they mostly remain throughout anaphase – this directly results in aneuploidy. Surprisingly, k-fibre stability is highly reduced in the absence of spindle actin. Conversely, targeted enrichment of spindle actin remarkably stabilises kinetochore-microtubule bundles, which also causes aneuploidy. This study has provided strong evidence that microtubules alone are insufficient to accurately separate chromosomes in mammalian oocytes (34). Instead, they are reinforced with actin to generate functional k-fibres that can pull chromosomes apart during meiosis. Cell biologists are today armed with continuously evolving actin probes (35) and it is high time we revisited spindle actin function in many other systems. Indeed, we begin this new decade with fresh reports of mitotic spindle actin existence in vertebrate cells (36, 37).

**8. CENP-A retention governs inheritance of centromere identity**

In addition to serving as sites of cohesion, centromeres specify chromosomal regions for kinetochore assembly. When DNA is replicated in dividing cells, each sister centromere receives equal portions of CENP-A nucleosomes. Soon after mitotic exit, newly synthesised CENP-A is assembled at centromeres by evicting 'place holder' histones (38). Presence of CENP-A nucleosomes critically defines where functional centromeres are located on chromosomes (39, 40). Retention of CENP-A in this way is thus a critical mark for centromere inheritance in dividing cells. But mammalian oocytes can remain arrested in meiotic prophase for months or even decades. How then are centromeres maintained and later inherited in the female germline? The answer came from a genetic study that knocked out CENP-A at very early stages of mouse development, before meiosis. Surprisingly, this does not reduce the amount of CENP-A at oocyte centromeres after prolonged meiotic prophase arrest. Consistently,

knock out oocytes can mature into eggs and form functional kinetochores that participate in chromosome alignment. Thus, CENP-A nucleosomes assembled in pre-meiotic stages are so stable during prolonged periods of arrest that they can maintain centromere identity throughout the fertile lifespan of a female (41). Indeed, deletion of CENP-A early in development has no notable effect on fertility – eggs are formed normally from CENP-A knock out mouse oocytes and produce healthy embryos when fertilised by wild-type sperm. A substantial addition to the field, this work showed how oocytes deal with their unconventionally long division programme to convey to the offspring where to find the centromeres.

**9. Selfish centromeres and spindle asymmetry cause chromosomal inequality**

According to Mendel's law of independent assortment, all chromosomes stand equal chance of transmission. No stranger to breaking rules, meiosis violates this mandate – meiotic drive, wherein selfish genetic elements are more likely to be retained in the newly formed egg, distorts expected chromosomal transmission ratios (42, 43). In oocytes, disparities in centromere size within homologue pairs were shown to underpin meiotic drive - stronger (larger) centromeres are preferentially kept inside the egg at the expense of weaker (smaller) centromeres that are discarded into the polar body (44). Seminal studies in the second half of the decade revealed exciting tactics of achieving chromosomal inequality. In hybrid mouse oocytes that contain homologous centromere pairs of unequal strength, bivalents where stronger centromeres are originally destined for elimination could be seen flipping right before anaphase (45) (Figure 4A). This last-ditch effort often results in binning of weaker centromeres along with a half-spindle that is intriguingly more tyrosinated. How this tubulin post-translational modification is linked to centromere size was meticulously demonstrated by a follow up study (46). Stronger centromeres recruit more Bub1 kinase, which catalyses histone phosphorylation for more potent recruitment of the microtubule depolymerising kinesin MCAK. Importantly, because MCAK preferentially depolymerises tyrosinated microtubules, its amplification destabilises microtubule attachments mainly on strong centromeres closer to the oocyte cortex. In this mouse model, unequal tubulin modification asymmetry occurs only when the spindle and chromosomes approach the cell surface. In this way, selfish (stronger) centromeres exploit cell surface-derived spindle asymmetry to detach from the polar body-bound half-spindle and reattach to the side that will be retained inside the egg. It is possible

that bivalent flipping very late in meiosis I is a last meiotic drive resort. Eggs from another, genetically distinct hybrid mouse model also preferentially retain stronger centromeres but do so by exploiting asymmetries that arise early in meiosis I (47) (Figure 4B). Here, meiosis begins with more microtubules and their organising centres on the side of the spindle that is destined for elimination. This early onset spindle asymmetry is proposed to promote bivalent flipping toward the egg side before spindle migration. In this model, spindle detachments that facilitate centromere re-orientation could be mediated by Aurora B/C kinases, although involvement of MCAK and other microtubule destabilisers cannot be excluded. Even though strategic details of meiotic drive appear distinct between these models, spindle asymmetry is a common denominator – independently of when during meiosis it arises, asymmetry promotes fascinating bivalent gymnastics to retain stronger centromeres inside the egg.

These studies were the first to provide molecular details of how centromere inequalities can enforce meiotic drive. Like many other innovative works of the decade, discoveries here too were heavily driven by high-resolution live imaging assays that continue to transform the field.

**10. Meiomaps reveal unconventional meiosis in human oocytes**

DNA exchange between homologues (crossover) by way of meiotic recombination underlies genetic diversity in sexually reproducing organisms. Crossovers bolster chromosome cohesion because they also serve to physically hold homologues together. Some crossovers are counterproductive, however. For instance, those that occur too close to centromeric regions are undesirable since they can interfere with centromeric cohesion (48-51). Consistently, defective DNA recombination events can generate unpaired homologues that contribute to oocyte aneuploidy (52, 53). However, the field still lacked a comprehensive picture of meiotic recombination landscapes and their association with oocyte and embryo aneuploidy. This gap was significantly narrowed by an elegant and original study that used genome-wide analysis to construct ‘meiomaps’ of crossover and segregation patterns during human female meiosis (54). SNP genotyping of chromosomes in all three products of meiosis (two polar bodies and an activated egg or an embryo) revealed previously unknown aspects of meiosis. Oocyte chromosomes experience generally higher rates of recombination than their male counterparts. Importantly, higher recombination

frequencies may protect oocytes against chromosome mis-segregation because aneuploid oocytes and embryos have significantly less recombination events than euploid ones. Strikingly, human oocytes can undergo a reverse mode of chromosomal segregation - sister chromatids are separated during meiosis I (Figure 3B) and non-sisters are segregated upon fertilisation. Interestingly, such peculiar meiosis does not invariably cause oocyte aneuploidy, a conundrum that can surely be solved by live imaging-based interrogation of reverse segregation. Finally, this work highlighted meiotic drive for recombinant chromatids in human eggs – non-recombined chromatids are more likely to be discarded into the polar body upon fertilisation. Curiously, stronger/larger centromeres can exploit spindle asymmetry for meiotic drive in mice (discussed earlier) (45, 47). Since larger centromeres, particularly on smaller chromosomes, can in principle reduce chromosomal regions available for beneficial crossovers, it would seem that centromere drive and recombinant chromatid drive are somewhat at odds. Equipped with advanced live imaging tools as well as recent (55) and forthcoming genome-wide association studies of meiosis, this is one open question the field is poised to answer in the new decade.

**Other advances of the decade**

The complex and diverse nature of oocyte meiosis means that no list can ever be extensive. Indeed, many pieces of work not discussed above have illuminated several key aspects of meiosis in the last decade. Programmed Rec8 removal experiments have revealed that chromosome cohesion is established before birth and is maintained without turnover (56). On the meiosis cell cycle front, it was shown that degradation of excess Cyclin B prevents premature destruction of CDK1-bound Cyclin B fractions for accurate chromosome segregation (57). Studies of oocyte genome stability have demonstrated that DNA damage blocks the production of fertilisable eggs by stalling meiosis I (58). This arrest is mediated by the spindle assembly checkpoint and its efficiency is affected by advancing maternal age (59, 60). Aurora kinases have been added to the oocyte's solutions to meiosis-specific problems – loss of bivalent stretching forces in meiosis I induces error correction by AURKB/C, which lengthens meiosis and allows time for the formation of proper kinetochore-microtubule attachments (61). AURKB also negatively regulates AURKC for accurate chromosome segregation whereas AURKC helps to maintain AURKA at spindle poles where it is needed for spindle morphology (62). Centromeric cohesion during anaphase is

protected by shugoshin (Figure 2B), which intriguingly is undetectable at centromeres during late anaphase. Cohesion maintenance during this oocyte-to-egg transition period was found to require the SUMO pathway, whose activity is enriched at centromeres in late anaphase I (63). In mouse oocytes, the role of actin filaments was also extended to positioning the oocyte nucleus during prophase (64) as well as reducing cortical tension, a prerequisite for asymmetric spindle positioning (65, 66). Our understanding of meiosis mechanisms in non-mammalian oocytes has also increased profoundly. During meiosis in starfish oocytes, a bias in dynein-driven spindle positioning helps to specifically discard older (mother) centrioles into polar bodies (67). In *Drosophila* oocytes, in contrast, downregulation of Polo kinase and disruption of PCM were found to be critical steps in centriole elimination before meiotic divisions, which is required for embryonic development (68). How do these oocytes then build spindles without centrioles? A new pathway of microtubule nucleation where kinesin-6 Subito/MKlp2 recruits gamma-tubulin complexes to chromosomal regions was shown to support spindle assembly (69). In *C. elegans*, where fertilisation occurs during meiosis, live imaging showed that F-actin prevents premature interactions between sperm microtubule asters and the oocyte meiotic spindle, which is needed for accurate completion of meiosis (70). Interestingly, a kinetochore-independent form of segregation (71) where inter-chromosomal microtubule arrays push meiotic chromosomes apart is evident in this system (71-73). Here, sumoylation-regulated assembly of a protein ring complex around the bivalents is required for correct chromosome segregation (74, 75).

**Concluding thoughts**

These discoveries have opened many new avenues in meiosis research, and we have much to anticipate in the new decade. The field is ready to benefit from gentle super-resolution microscopy techniques suitable for long-term live imaging of meiotic events at unprecedented detail. The first live imaging studies of human oocytes have highlighted problematic modes of chromosome segregation (76, 77). New human oocyte donation-based research programmes are expected to illuminate spindle organisation and chromosome separation mechanisms in human oocytes with high developmental capacity. Large-scale electron tomography recently revealed ultrastructural details of meiotic spindle organisation in *C. elegans* oocytes (72, 73). This approach will be instrumental in thoroughly understanding mammalian oocyte

spindle architecture, possibly forcing us to revise some models of chromosome segregation. The meiosis field is yet to benefit from biochemical experiments that have advanced mitosis research. These challenges can be overcome, and we can perhaps expect to gain mechanistic details of meiosis-specific cytoskeletal crosstalk through *in vitro* reconstitution assays. Finally, a growing number of genome-wide association studies are likely to accelerate identification of new genes involved in oocyte aneuploidy and human infertility. Powerful oocyte protein degradation assays such as TRIM-Away (78) should facilitate probing the function of new candidate genes.

In conclusion, while there is still plenty to learn, the field is steadily pacing toward a milestone where phrases like 'poorly understood' should be used with caution.

**Summary points**

- Chromosome segregation errors are a leading cause of oocyte aneuploidy.
- Premature cohesin loss in aging oocytes contributes to chromosome segregation errors and the maternal age effect.
- Fusion of sister kinetochores ensures their co-orientation and underpins homologous chromosome segregation in meiosis I.
- Actin-based transport and actin-microtubule coordination in meiosis prevent oocyte aneuploidy.
- Asymmetries in spindle tubulin and its post-translational modification underlie meiotic drive in oocytes.

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## FIGURE LEGENDS

**FIGURE 1. Meiosis in mammalian oocytes.** Immature oocytes are arrested in prophase of meiosis I. Depending on the species, this arrest can last from years to decades. When meiosis resumes, the nuclear envelope disassembles. This releases the homologous chromosomes into the cytoplasm where there are captured by microtubules of the assembling meiotic spindle. The chromosomes are then transported to the oocyte surface and separated in anaphase I. Here, half of them are discarded into a small polar body while the other half (sister chromatids) are again rearranged on the meiosis II spindle. In this metaphase II arrested state, the egg awaits fertilisation, upon which the sister chromatids are separated in anaphase II and a genetically unique zygote is formed.

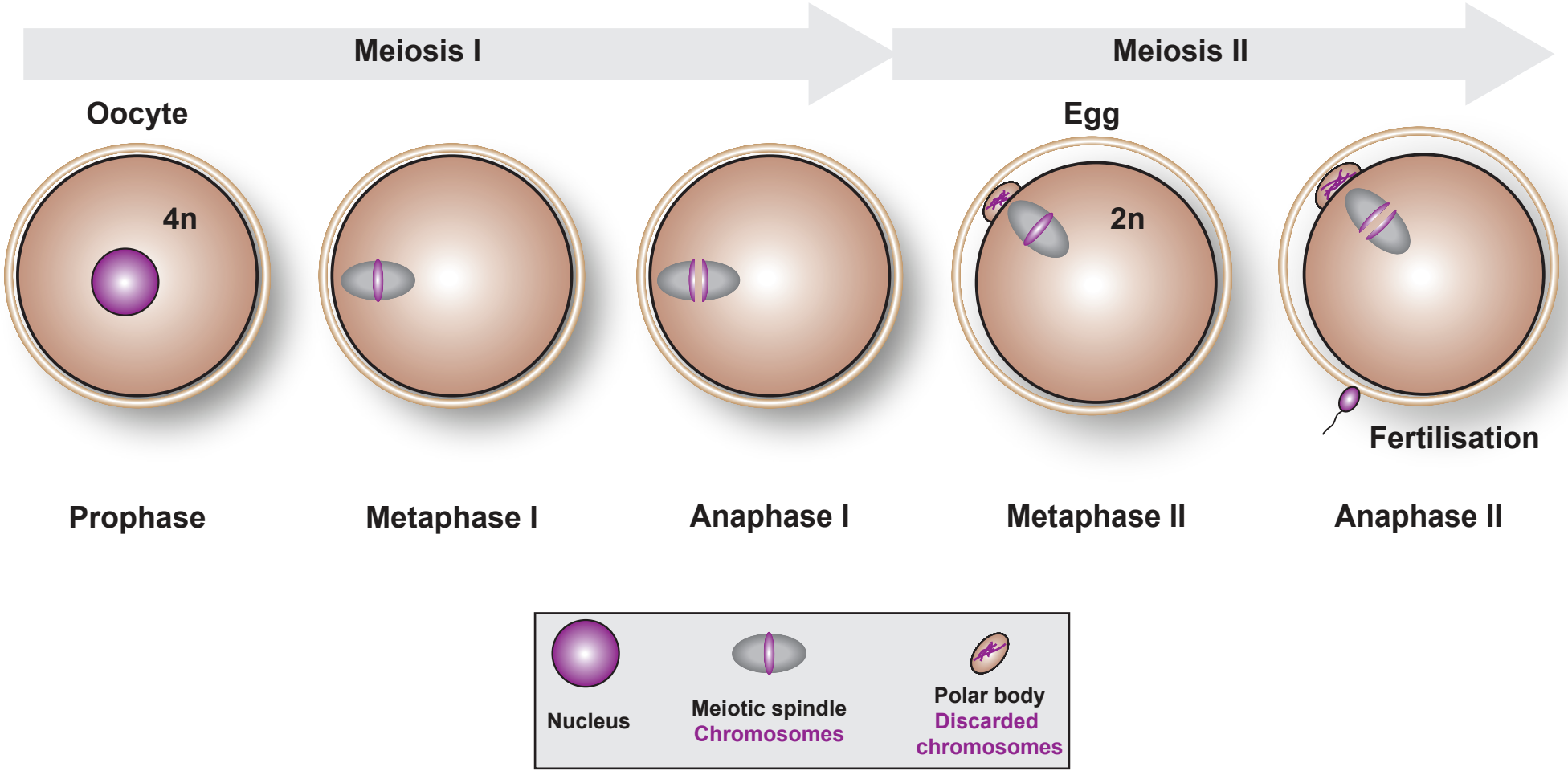
**FIGURE 2. The principle of meiotic chromosome segregation. A.** Homologous recombination during meiosis yields recombinant chromatids within each homologue pair (presented as a switch in colour along chromosomes). In meiosis I, homologous chromosomes (consisting of sister chromatids) form a bivalent structure that is held together by cohesive forces (presented as orange rings) along the chromosomes' arms and their centromeres/kinetochores (light blue circles). Kinetochores of sister chromatids (sister kinetochores) are normally fused and co-orient in meiosis I – they face the same spindle pole (orange blobs) (highlight 2). When fully aligned on the spindle, attachment of each sister chromatid pair to k-fibres (dark green lines) originating from opposite poles generates stretching forces on the bivalent. Loss of bivalent stretching activates the spindle assembly checkpoint (60). **B.** In anaphase I, homologous chromosomes are separated while sister chromatids are kept together. This is achieved by selective removal of cohesion along chromosome arms. Centromeric cohesion in early anaphase I is protected from removal by a mechanism that involves the protein shugoshin. **C.** In meiosis II, sister kinetochores are bi-oriented and connected to k-fibres originating from opposite spindle poles. Their separation in anaphase II requires removal of remaining cohesion from the centromeres.

**FIGURE 3. Unfused meiosis I sister kinetochores are sources of aneuploidy. A.** Bivalents in oocytes of younger women generally contain mechanically fused sister kinetochores – the coupling is so tight that they appear to be connected to a joint k-

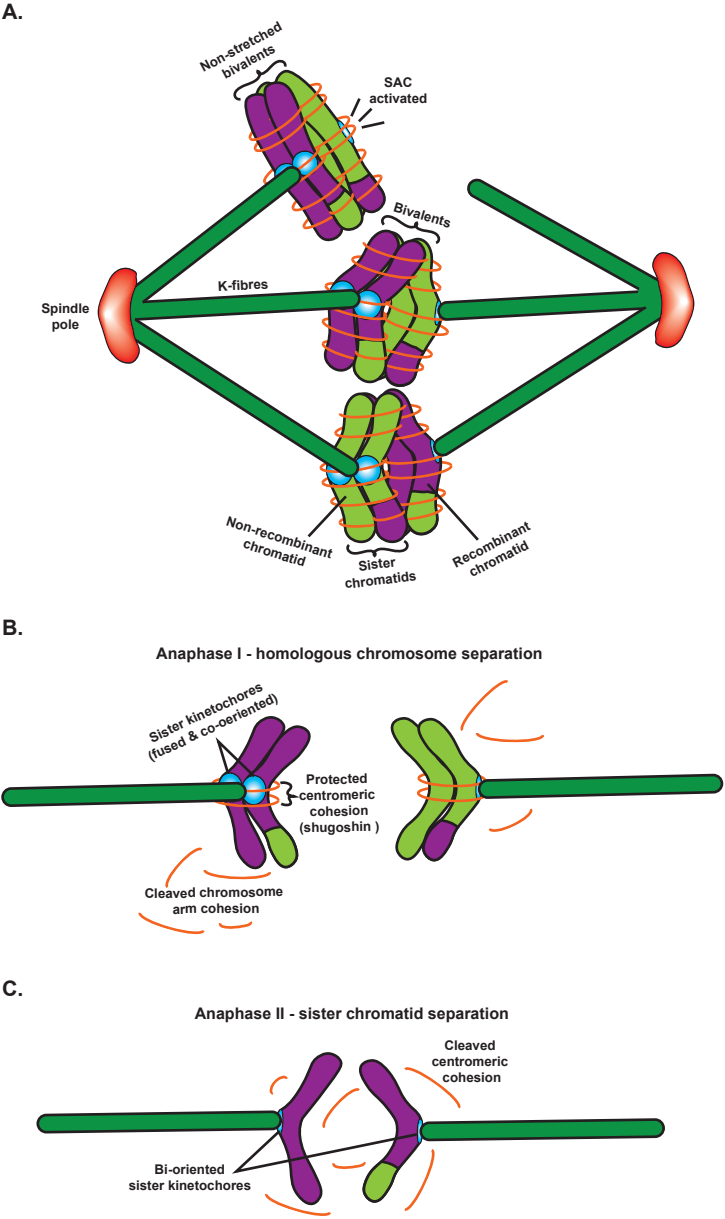
fibre bundle. Deterioration of cohesion with increasing maternal age causes increased spacing between or complete splitting of sister kinetochores – in some cases, they are so far apart that they can connect to distinct k-fibre bundles (highlight 3). **B.** Extreme sister kinetochore splitting combined with unique geometry of some human oocyte chromosomes can lead to sister kinetochore/chromatid bi-orientation in meiosis I. This can lead to sister chromatid separation in anaphase I. Indeed, this reverse mode of chromosome segregation has been observed in human oocytes (highlight 10). Sister kinetochores are presented as same-coloured circles (light blue or light yellow).

**FIGURE 4. Spindle asymmetry underpins centromere drive.** Two studies have addressed underlying mechanisms of centromere drive using different hybrid mouse models (highlight 9). **A.** Centromere drive is achieved by exploiting asymmetric spindle positioning feature of oocyte meiosis. Proximity of the chromosomes to the oocyte cortex late in meiosis I triggers cell surface-based signals that increase overall microtubule tyrosination on the cortical side of the spindle. Stronger centromeres can recruit more Bub1 kinase, which in turn facilitates recruitment of MCAK, a kinesin that preferentially depolymerises tyrosinated microtubules. In this way, k-fibres of cortically positioned stronger centromeres are preferentially destabilised to promote bivalent flipping before anaphase onset. **B.** In the second hybrid model, asymmetry is more inherent – the spindle assembles with more clusters of acentriolar microtubule organising centres (aMTOC) on the cortical side. Consistently, more microtubules are present on this side of the spindle. Here, bivalent flipping and stronger centromere retention occurs prior to asymmetric spindle positioning, early in meiosis I. This is dependent on Aurora B/C, although participation on MCAK and BubI cannot be excluded.

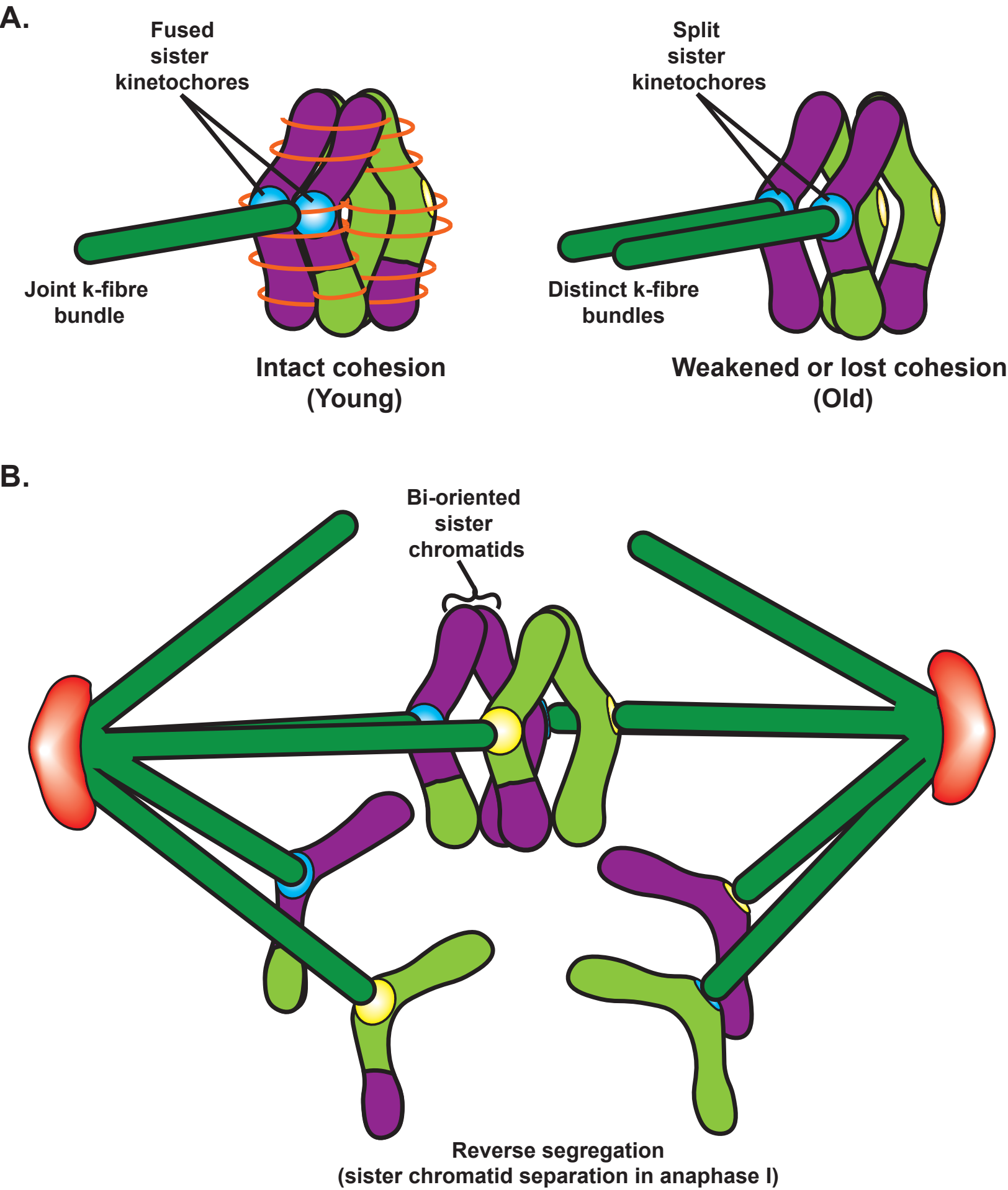
# FIGURE 1


Meiosis I
Meiosis II
Oocyte
4n
Egg
2n
Fertilisation
Prophase
Metaphase I
Anaphase I
Metaphase II
Anaphase II
Nucleus
Meiotic spindle
Chromosomes
Polar body
Discarded
chromosomes

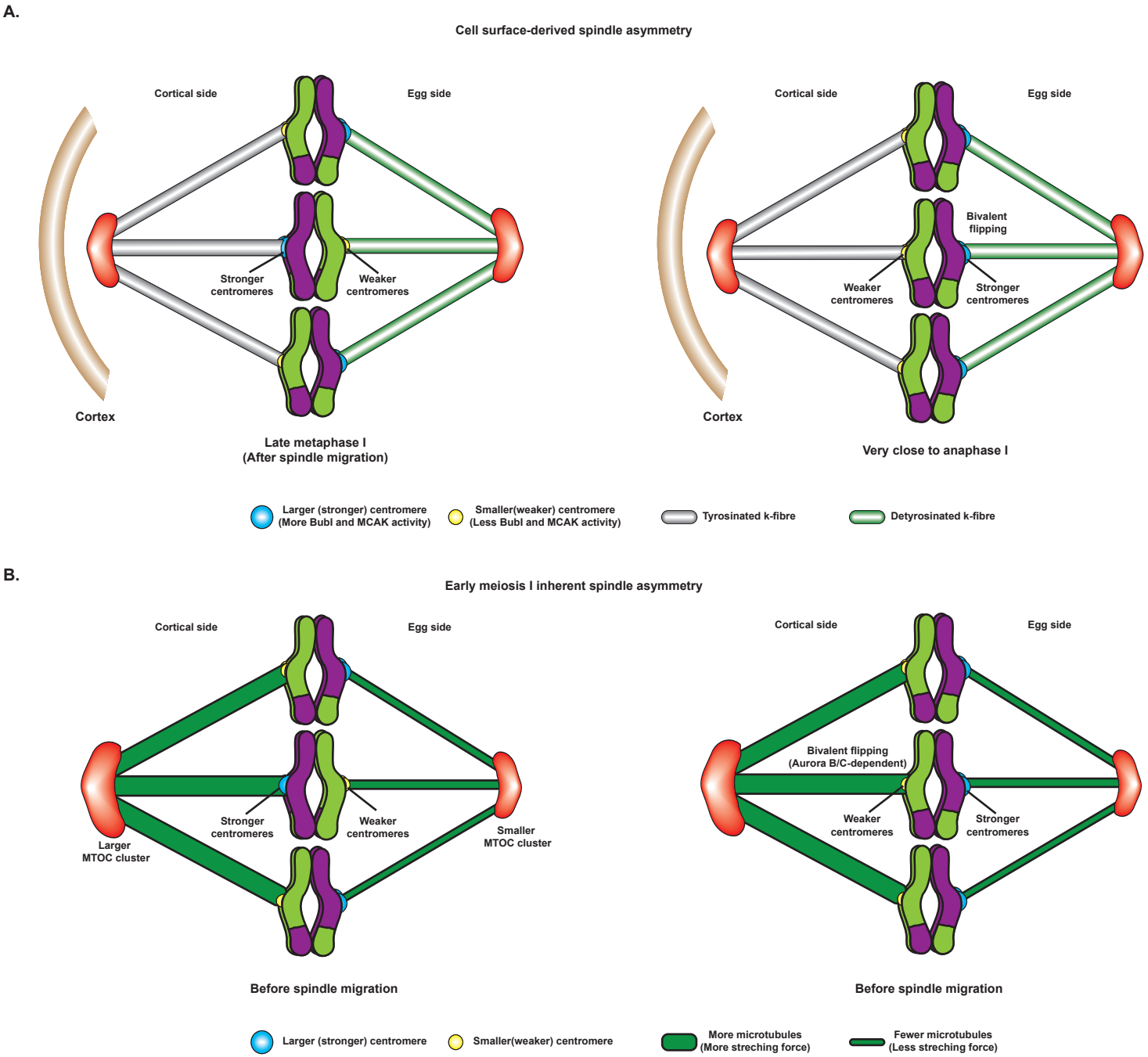
# FIGURE 2


A.
Non-stretched bivalents
SAC activated
Bivalents
K-fibres
Spindle pole
Non-recombinant chromatid
Sister chromatids
Recombinant chromatid
B.
Anaphase I - homologous chromosome separation
Sister kinetochores (fused & co-oeriented)
Protected centromeric cohesion (shugoshin )
Cleaved chromosome arm cohesion
C.
Anaphase II - sister chromatid separation
Cleaved centromeric cohesion
Bi-oriented sister kinetochores

# FIGURE 3


A.
Fused sister kinetochores
Joint k-fibre bundle
Intact cohesion (Young)
Split sister kinetochores
Distinct k-fibre bundles
Weakened or lost cohesion (Old)
B.
Bi-oriented sister chromatids
Reverse segregation (sister chromatid separation in anaphase I)

# FIGURE 4


A.
Cell surface-derived spindle asymmetry
Cortical side
Egg side
Stronger centromeres
Weaker centromeres
Cortex
Late metaphase I
(After spindle migration)
Cortical side
Egg side
Bivalent flipping
Weaker centromeres
Stronger centromeres
Cortex
Very close to anaphase I
Larger (stronger) centromere
(More Bubl and MCAK activity)
Smaller(weaker) centromere
(Less Bubl and MCAK activity)
Tyrosinated k-fibre
Detyrosinated k-fibre
B.
Early meiosis I inherent spindle asymmetry
Cortical side
Egg side
Stronger centromeres
Weaker centromeres
Larger MTOC cluster
Smaller MTOC cluster
Before spindle migration
Cortical side
Egg side
Bivalent flipping (Aurora B/C-dependent)
Weaker centromeres
Stronger centromeres
Before spindle migration
Larger (stronger) centromere
Smaller(weaker) centromere
More microtubules
(More streching force)
Fewer microtubules
(Less streching force)